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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 7/10, 15/06, A61K 37/02, 39/395

A1

(11) International Publication Number: WO 94/12531

(43) International Publication Date: 9 June 1994 (09.06.94)

(21) International Application Number: PCT/US93/11110

(22) International Filing Date: 19 November 1993 (19.11.93)

(30) Priority Data:
07/980,527 20 November 1992 (20.11.92) US

(60) Parent Application or Grant
(63) Related by Continuation
US 07/980,527 (CON)
Filed on 20 November 1992 (20.11.92)

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(81) Designated States: AU, BB, BG, BR, BY, CA, CZ, FI, HU, JP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ANTAGONISTS OF HUMAN GAMMA INTERFERON

(57) Abstract

Antagonists of human IFN-γ are provided which are based upon a critical region of the human IFN-γ receptor. Examples of such antagonists, which mimic, comprise or specifically bind to the critical region or the receptor, are polypeptides and antibodies or fragments thereof. Also provided are compositions and methods for inhibiting the biological activity of human IFN-γ.

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ANTAGONISTS OF HUMAN GAMMA INTERFERON

TECHNICAL FIELD

This invention relates to antagonists of human gamma interferon that are based upon a critical region of the human gamma interferon receptor.

BACKGROUND OF THE INVENTION

Gamma interferon (IFN-γ) is a cytokine produced by activated helper T cells, one of the most characteristic activities of which is the upregulation of Major Histocompatibility Complex (MHC) class II gene expression in macrophages, mature B cells and T cells. The expression of class II antigens is a hallmark of antigen-presenting cells. IFN-γ is also known to upregulate the expression of class II antigens in cells that are not primary antigen-presenting cells, such as epithelial cells, fibroblasts, astrocytes, endothelial and smooth muscle cells. The upregulation of class II antigens in these cell types is often correlated with the development of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.

Although the mechanism by which IFN-γ exerts its effects on cells is not understood, it is known that it binds to specific cellular receptors [Langer *et al.*, *Immunology Today* *9*:393 (1988)]. Aguet *et al.* [*Cell* *55*:273 (1988)] have cloned and sequenced a gene for a IFN-γ receptor. The molecular weight of the encoded protein deduced from the sequence is consistent with the molecular weight of a IFN-γ isolated from human placenta [Calderon *et al.*, *Proc. Natl. Acad. Sci.* USA *85*:4837 (1988)]. Furthermore, the human IFN-γ receptor has been expressed in a biologically active form in Chinese

hamster ovary (CHO) cells. The extracellular domain of the high affinity IFN-γ receptor has an amino acid sequence defined in the Sequence Listing by SEQ ID NO: 1.

Because IFN-γ acts at specific cellular receptors and is implicated in autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, there is a need for agents that inhibit the binding of such interferon to cellular receptors.

SUMMARY OF THE INVENTION

The present invention fills this need by providing IFN-γ antagonists, compositions and methods for inhibiting the biological activity of human IFN-γ.

More particularly, this invention provides antagonists of human IFN-γ that mimic, comprise or specifically bind to an amino acid sequence of a region of the human IFN-γ receptor, which region has an amino acid sequence defined by the sequence of SEQ ID NO: 2.

This invention further provides methods for inhibiting the biological activity of human IFN-γ comprising contacting human IFN-γ or cells bearing receptors for human IFN-γ with an antagonist of human IFN-γ that mimics, comprises or specifically binds to an amino acid sequence of a region of the human IFN-γ receptor, which region has an amino acid sequence defined by the sequence of SEQ ID NO: 2.

In one embodiment of this invention, the antagonists are polypeptides which contain a core sequence defined by SEQ ID NO: 3 and comprise from about 22 to 48 amino acid residues of the amino acid sequence defined by SEQ ID NO: 4, wherein in both sequences residues represented as Xaa at positions 2 and 3 can be Tyr or Val and Ser or Cys, respectively, and the sulfhydryl groups of Cys residues in the

polypeptides can be free or blocked by a sulfhydryl blocking group.

In another embodiment the antagonists are antibodies or fragments thereof that specifically bind to an epitope of a polypeptide having an amino acid sequence defined by part or all of the sequence of SEQ ID NO: 2, and to the human IFN-γ receptor.

In still another embodiment the antagonists are anti-idiotypic antibodies or fragments thereof produced against an antibody or a fragment thereof that specifically binds to an epitope of a polypeptide having an amino acid sequence defined by part or all of the sequence of SEQ ID NO: 2, and to the human IFN-γ receptor.

BRIEF DESCRIPTION OF THE FIGURES

This invention can be more readily understood by reference to the accompanying Figures, in which:

Fig. 1 is a graphical representation of the inhibition of IFN-γ-induced expression of HLA/DR antigen on Colo 205 cells by a polypeptide antagonist having an amino acid sequence defined by SEQ ID NO: 5, wherein the sulfhydryl group of the cysteine residue at position 3 was blocked by an acetamidomethyl group.

Fig. 2 is a graphical representation of the binding to human IFN-γ of a polypeptide antagonist having an amino acid sequence defined by SEQ ID NO: 5, wherein the sulfhydryl group of the cysteine residue at position 3 was blocked by an acetamidomethyl group. The amount of IFN-γ bound to the polypeptide coated onto the wells of a microtiter plate is shown as a function of absorbance at 405 nm.

DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirety by reference. All amino acid sequences disclosed follow the normal convention, with amino termini on the left and the carboxyl termini on the right. Standard three-letter abbreviations are used for the amino acid residues in the sequences.

As used herein, the human "IFN-γ receptor" means a protein which (a) has an amino acid sequence substantially as defined in the Sequence Listing by SEQ ID NO: 1 and (b) has biological activity that is common to the native IFN-γ receptor and which binds to human IFN-γ.

The antagonists of this invention can potentially be used to treat any medical condition caused by IFN-γ, such as autoimmune disease. They can also be used to elucidate the mechanism of action of IFN-γ and can be used as part of a screening system to identify agonists and/or other antagonists of IFN-γ.

As used herein, the term "antagonist" is defined as a substance that blocks or inhibits the binding of human IFN-γ to cellular receptors and thereby inhibits one or more of the known biological activities of IFN-γ. Depending upon the particular antagonist, such inhibition may involve binding of an antagonist to IFN-γ or to the IFN-γ receptor.

It has surprisingly been found that there is a critical region of the human IFN-γ receptor that is apparently involved in IFN-γ/receptor interactions. Agents that mimic or comprise a subsequence of this critical region, and antibodies against the region or anti-idiotypic antibodies against such antibodies, can inhibit the interaction between IFN-γ and the receptor.

The critical region of the human IFN-γ receptor has an amino acid sequence defined by the sequence of residues 120 to 167 of SEQ ID NO: 1. Surprisingly, it has been found that polypeptides containing a core sequence based upon the sequence of residues 120 to 141 of SEQ ID NO: 1 are effective antagonists of IFN-γ. The present invention provides such polypeptides, as well as compounds that can mimic such polypeptides.

From the foregoing, it should be clear that any polypeptide comprising the core sequence defined by the sequence of residues 120 to 141 of SEQ ID NO: 1 (which is also the sequence defined by SEQ ID NO: 3) will inhibit the binding of IFN-γ to cellular receptors and, hence, biological activity. Thus this invention encompasses not only the above-mentioned polypeptides, but also others that are intermediate in length (i.e., those which contain in addition to the 22-residue core sequence of SEQ ID NO: 3, one or more of the other amino acid residues shown in SEQ ID NO: 4) and inhibit the binding and biological activity of IFN-γ.

It should be noted that some variation is present in the sequences of SEQ ID NO: 3 and SEQ ID NO: 4. Residues represented as Xaa at positions 2 and 3 in both sequences can be Tyr or Val and Ser or Cys, respectively. Any or all of the sulfhydryl groups of the cysteine residues in the polypeptides can be free or covalently blocked by any of the known sulfhydryl blocking groups, such as the acetamidomethyl group. Other reagents that can be used to block sulfhydryl groups include, e.g., alkylating agents, such as iodoacetate or iodoacetamide; anhydrides such as maleic or succinic anhydride; and DTNB [5,5'-dithiobis(2-nitrobenzoic acid)].

Although the inhibitory effects of an exemplary antagonist are demonstrated below using COLO-205 cells, the antagonists of this invention will inhibit the binding of IFN-γ

to any cells bearing IFN-γ receptors, such as B cells, T cells, eosinophils, smooth muscle cells, promyelocytes, macrophages, erythroid cells, monocytes and granulocytes. For example, Daudi cells, a well-characterized B lymphoblast cell line derived from a Burkitt lymphoma patient which are available from the American Type Culture Collection under Accession No. CCL 213, can also be used. Effects of the antagonists can be observed by measuring inhibition of the binding of ^{125}I-labeled IFN-γ to cellular receptors on such cells. Other cell lines can also be used for this purpose, such as U-937 human lymphoma line (ATCC CRL 1593). The radiolabeled IFN-γ can be prepared by standard methods.

The polypeptide antagonists of the invention can be synthesized by a suitable method such as by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid phase peptide synthesis as described, e.g., by Merrifield [*J. Am. Chem. Soc. 85*:2149 (1963); *Science 232*:341 (1986)] and Atherton *et al.* (*Solid Phase Peptide Synthesis: A Practical Approach*, 1989, IRL Press, Oxford). The synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the polypeptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise polypeptide synthesis. Included are acyl type protecting groups (e.g., formyl, trifluoroacetyl, acetyl), aromatic urethane type

protecting groups [e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl (Fmoc)], aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (Boc), isopropyloxycarbonyl, cyclohexyloxycarbonyl) and alkyl type protecting groups (e.g., benzyl, triphenylmethyl). The preferred protecting group is Boc. The side-chain protecting groups for Tyr include tetrahydropyranyl, tert-butyl, trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl and cyclohexyl. The preferred side-chain protecting group for Asp is cyclohexyl. The side-chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl and Cbz. The preferred protecting group for Thr and Ser is benzyl. The side-chain protecting groups for Arg include nitro, Tos, Cbz, adamantyloxycarbonyl and Boc. The preferred protecting group for Arg is Tos. The side-chain amino group of Lys may be protected with Cbz, 2-Cl-Cbz, Tos or Boc. The 2-Cl-Cbz group is the preferred protecting group for Lys.

The side-chain protecting groups selected should remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups should also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished polypeptide.

Solid phase synthesis is usually carried out from the carboxy-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting polypeptide will have a free carboxyl group at the C-terminus.

Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting polypeptide will have a carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by Stewart *et al.*, *Solid Phase Peptide Synthesis* (2nd Edition), Pierce Chemical Co., Rockford, IL., 1984.

The C-terminal amino acid, protected at the side-chain if necessary and at the alpha-amino group, is coupled to the benzhydrylamine resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide and carbonyldiimidazole. Following the attachment to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0° and 25°C. Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

Various activating agents can be used for the coupling reactions including DCC, N,N'-diisopropylcarbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) and DCC-hydroxybenzotriazole (HOBt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH_2Cl_2 or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser *et al.*, *Anal. Biochem.*, *34*:595 (1970). In cases where incomplete coupling is found, the coupling reaction is repeated. The coupling reactions can be

performed automatically with commercially available instruments.

After the entire assembly of the desired polypeptide, the polypeptide-resin is cleaved with a reagent such as liquid HF for 1-2 hours at 0°C, which cleaves the polypeptide from the resin and removes all side-chain protecting groups. A scavenger such as anisole is usually used with the liquid HF to prevent cations formed during the cleavage fom alkylating the amino acid residues present in the polypeptide. The polypeptide-resin may be deprotected with TFA/dithioethane prior to cleavage if desired.

Side-chain to side-chain cyclization on the solid support typically requires the use of an orthogonal protection scheme which enables selective cleavage of the side-chain functions of acidic amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-fluorenylmethyl (Fm) protecting group for the side-chain of Asp and the 9-fluorenylmethyloxy-carbonyl (Fmoc) protecting group for the side-chain of Lys can be used for this purpose. In these cases, the side-chain protecting groups of the Boc-protected polypeptide-resin are selectively removed with piperidine in DMF. Cyclization is achieved on the solid support using various activating agents including DCC, DCC/HOBt or BOP. The HF reaction is carried out on the cyclized polypeptide-resin as described above.

Recombinant DNA methodology can also be used to prepare polypeptide antagonists. See, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 1989, Cold Spring Harbor Press, Cold Spring Harbor, New York. The known genetic code, tailored if desired for more efficient expression in a given host organism, can be used to synthesize oligonucleotides encoding the desired amino acid sequences. The phosphoramidite solid support method of Matteucci *et al.* [*J. Am. Chem. Soc. 103*:3185 (1981)], the method of Yoo *et al.*

[*J. Biol. Chem. 764*:17078 (1989)], or other well known methods can be used for such synthesis.

The resulting oligonucleotides can be inserted into an appropriate vector and expressed in a compatible host organism. Alternatively, standard molecular biology techniques can be used to permit engineering of an appropriate gene for efficient expression, including tandemly repeated segments having convenient protease sites for later cleavage and processing.

The polypeptides can be purified using HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution or other known methods.

The present invention also encompasses polypeptide analogs and mimetics, as well as other polypeptides comprising amino acid sequences which differ slightly from the sequences defined above. For example, this invention also includes modifications of the polypeptide antagonists which have undergone conservative amino acid substitution, deletion and or addition, as long as the modified polypeptides retain the ability to bind to and thereby inhibit the biological activity of IFN-γ. Examples of of the most frequently observed amino acid substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu and Asp/Gly, and vice versa. Polypeptide antagonists produced in prokaryotic expression systems may also contain an additional N-terminal methionine residue, as is well known in the art.

As used herein, the terms "mimetic" and "analog" include polypeptides, organic compounds or peptidomimetics which adopt the same characteristics as the polypeptide antagonists. Included are molecules which adopt a portion of

the same physical structure, contain a portion of the same epitope, or adopt a secondary structure and binding conformation similar to those of a polypeptide antagonist.

The mimetics and analogs include organic gamma and beta turn mimetics [Sato *et al.*, *Biochem. Biophys. Res. Commun. 187*:999 (1992); Kahn *et al.*, *Tetrahedron Letters 30*:2317 (1989)], alpha helix and beta sheet mimetics [Regan *et al.*, *Science 241*:974 (1988)], and conformationally-restricted analogs [Kessler *et al.*, *Intl. J. Pep. Protein Res. 32*:183 (1988); Dutta et al., *Biochem. Biophys. Res. Commun. 159*:1114 (1989)], as could be obtained, e.g., by cysteine bonds and glutamate-lysine bonds [Marqusee *et al.*, *Proc. Natl. Acad. Sci.* USA *84*:8898 (1987); Olivera *et al.*, *J. Biol. Chem. 266*:22067 (1991)]. In addition, incorporation of unnatural amino acids such as D-methyl, N-methyl and alpha methyl derivatives (Dutta *et al.*, *supra*) and non-peptidic structural elements [Rajashekhar *et al.*, *J. Biol. Chem. 261*:13617 (1986)] are also contemplated by this invention.

The antagonists of this invention should preferably produce at least about 25% inhibition of a biological activity of IFN-γ in cells bearing IFN-γ receptors. More preferably, the degree of inhibition will be at least about 75% and, most preferably, at least about 95%.

The IFN-γ antagonists of this invention also include antibodies or fragments thereof which specifically bind to the polypeptides and to the human IFN-γ receptor. By binding to the receptor, these antibodies and antibody fragments also inhibit the binding, and hence the biological activity, of human IFN-γ.

The polypeptide antagonists, which can be used as antigens to produce such antibodies and fragments, comprise one or more antigenic determinants (epitopes) against which

the production of antibodies can be elicited. As is well known in the art, such epitopes generally contain at least about 5 amino acid residues [Ohno *et al.*, *Proc. Natl. Acad. Sci.* USA *82*:2945 (1985)]. Antibodies produced using the polypeptide antagonists as antigens will specifically bind to an epitope on the polypeptides and to the human IFN-γ receptor as well.

The use and generation of fragments of antibodies is well known, e.g., Fab fragments [Tijssen, *Practice and Theory of Enzyme Immunoassays* (Elsevier, Amsterdam, 1985)], Fv fragments [Hochman *et al.*, *Biochemistry 12*:1130 (1973); Sharon *et al.*, *Biochemistry 15*:1591 (1976); Ehrlich *et al.*, U.S. Patent No. 4,355,023] and antibody half molecules (Auditore-Hargreaves, U.S. Patent No. 4,470,925).

Polyclonal antibodies can be produced by immunizing a host animal such as a rabbit, rat, goat, sheep, mouse, etc. with one of the polypeptides. Preferably, one or more booster injections are given after the initial injection, to increase the antibody titer. Blood is then drawn from the animal and serum is prepared and screened by standard methods such as enzyme-linked immunosorbent assay (ELISA) using the polypeptides as the antigen. The use of monoclonal antibodies, however, is preferred.

Hybridomas and monoclonal antibodies can be produced by standard methods [Kohler *et al.*, *Nature 256*:495 (1975); Kohler *et al.*, *Eur. J. Immunol. 6*:511 (1976)], using one of the polypeptide antagonists as the antigen. Preferably, the immunogenicity of the polypeptides is increased by combination with an adjuvant and/or by conversion to a larger form prior to immunization of a suitable host animal.

A wide variety of suitable adjuvants is well known in the art. The immunogenicity of the polypeptides can also be enhanced by using standard methods to cross-link the

polypeptides or to couple them to an immunogenic carrier molecule such as keyhole limpet hemocyanin or a mammalian serum protein such as human or bovine gammaglobulin, or human, bovine or rabbit serum albumin. Preferably, but not necessarily, the protein carrier will be foreign to the host animal in which antibodies against the polypeptides are to be elicited.

This invention also provides anti-idiotypic antibodies or fragments thereof which are directed against the above-mentioned antibodies or antibody fragments. Such anti-idiotypic antibodies mimic or act like the original polypeptide antagonist antigen (see, e.g., U.S. Patent No. 4,731,237 to Regan *et al.*). Like the IFN-γ receptor itself, these antibodies are presumed to bind specifically and directly to IFN-γ.

Such anti-idiotypic antibodies are prepared by vaccinating an animal with an antibody (polyclonal or monoclonal) against a polypeptide of the present invention. They may be recovered as a whole polyclonal antiserum or as an IgG or other fraction thereof, or as monoclonal antibodies produced by cloned hybridomas.

Once a hybridoma producing a desired monoclonal antibody is obtained, the above-mentioned antibody fragments can be made.

Alternatively, DNA encoding the antibody can be cloned and sequenced, and techniques can be used to produce interspecific monoclonal antibodies wherein the binding region of one species is combined with a non-binding region of the antibody of another species [Liu *et al.*, *Proc. Natl. Acad. Sci.* USA *84*:3439 (1987)]. For example, the CDRs from a rodent monoclonal antibody can be grafted onto a human antibody, thereby "humanizing" the rodent antibody [Riechmann *et al.*,

Nature 332:323 (1988)]. More particularly, the CDRs can be grafted into a human antibody variable region with or without human constant regions. Such methodology has been used, e.g., to humanize a mouse monoclonal antibody against the p55 (Tac) subunit of the human interleukin-2 receptor [Queen *et al.*, *Proc. Natl. Acad. Sci.* USA *86*:10029 (1989)]. Fragments of such humanized antibodies can also be made.

Once the CDRs of the heavy and light chains of the monoclonal antibody have been identified, such sequence information can be used to design non-peptide mimetic compounds which mimic the functional properties of the antibody. Methods for producing such mimetic compounds have been described, e.g., by Saragovi *et al.* [*Science 253*:792 (1991)]. CDR sequence information can also be used to produce single-chain binding proteins comprising linked CDRs from the light and/or heavy chain variable regions, as described by Bird *et al.* [*Science 242*:423 (1988)], or biosynthetic antibody binding sites (BABS), as described by Huston *et al.* [*Proc. Natl. Acad. Sci.* USA *85*:5879 (1988)]. Single-domain antibodies comprising isolated heavy-chain variable domains [Ward *et al.*, *Nature 341*:544 (1989)] can also be prepared using the sequence information.

Because of their smaller size and reduced immunogenicity, the antibody-based IFN-γ antagonists used in this invention are preferably antibody fragments, BABS, mimetic compounds or single-domain antibodies. The use of humanized antibody sequences is also preferred.

Pharmaceutical compositions can be prepared using one or more of the IFN-γ antagonists. Such compositions, which can be used to treat any IFN-γ-related disease, can be prepared by admixing an effective amount of one or more of the antagonists and a physiologically acceptable carrier.

Useful pharmaceutical carriers can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient. Sterile water, alcohol, fats, waxes, and inert solids may be included in a carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agents) may also be incorporated into the pharmaceutical composition. Generally, compositions useful for parenteral administration of such drugs are well known; e.g. *Remington's Pharmaceutical Science*, 15th Ed. (Mack Publishing Company, Easton, PA, 1980). Single-dose packaging will often be preferred, e.g., in sterile form.

Alternatively, compositions of the invention may be introduced into a patient's body by implantable drug delivery systems [Urquhart *et al., Ann. Rev. Pharmacol. Toxicol. 24*:199 (1984)]. Such carriers are well known to those skilled in the art. The antagonists can also be incorporated into liposomes, or delivered by standard gene therapy techniques, including, e.g., direct DNA injection into tissues, the use of recombinant viral vectors and implantation of transfected cells. See, e.g., Rosenberg, *J. Clin. Oncol. 10*:180 (1992).

Determination of the appropriate dosage of an antagonist for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages that are less than optimum. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

The amount and frequency of administration of the antagonists and the pharmaceutically acceptable salts thereof will be regulated according to the judgment of the attending clinician, taking into account such factors as age, condition and

size of the patient and severity of the symptom(s) being treated.

EXAMPLE

The present invention can be illustrated by the following, non-limiting example. Unless otherwise specified, percentages given below for solids in solid mixtures, liquids in liquids, and solids in liquids are on a wt/wt, vol/vol and wt/vol basis, respectively.

Reagents and Cells

Recombinant human IFN-γs A and D [specific activity about 5 x 10^6 units/mg; Seelig *et al.*, *Biochemistry* 27:1981 (1988)] were prepared and purified from transformed *E. coli*, essentially as described in U.S. Patent No. 4,751,078.

COLO-205 cells (ATCC CLL 222) were used to measure the induction by the interferon of class II major histocompatibility antigens (HLA-DR). The presence of the antigens on the cells was detected by Enzyme-Linked Immunosorbent Assay (ELISA) using a mouse monoclonal anti-HLA-DR antibody (Becton-Dickinson Catalog No. 7360) in conjunction with a peroxidate-labeled goat anti-mouse IgG. Color produced using 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS; Kirkegaard & Perry Labs., Inc., Gaithersburg, MD) was measured spectrophotometrically at 405 nm.

General Methods

Protein determinations were carried out by the method of Lowry *et al.* [*J. Biol. Chem. 193*:265 (1951)] using bovine serum albumin as a standard. Polypeptide

concentrations were determined by amino acid analysis using gas phase HCl and 1 hour incubation at 150°C.

Rabbit or mouse antibodies were screened for specific binding of antigens using a direct solid phase ELISA at room temperature. A 96-well microtiter plate (NUNC, Intermed, Denmark) was coated with 100 µl of antigen per well for 1 hour at room temperature. The plate was washed 5 times with tris-buffered saline (TBS), pH 7.5, containing 0.05% TWEEN 20 (polyoxethylenesorbitan monolaurate). The plate was subsequently blocked with 1% bovine serum albumin (BSA) for 1 hour, washed 5 times with TBS, and coated with 2.5 ng of horseradish peroxidase-conjugate goat anti-rabbit IgG, or goat anti-mouse IgG.

Following incubation for 1 hour, the plate was washed 5 times with TBS and developed by adding either 2,2'-Azino-bis [3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) or 3,3',5,5'-Tetramethylbenzidine (TMB) and hydrogen peroxide to each well. Color development was stopped after 20 minutes by adding a solution containing sulfuric for TMB or sodium dodecylsulfate for ABTS, and the samples were read at 405 and 450 nm for ABTS and TMB, respectively, using a Molecular Devices ELISA reader.

Immunosorbent assays were carried out on polypeptides immobilized on pins as follows. The pins were blocked for 1 hour by inverting the pins onto a standard 96-well microtiter plate and incubating in phosphate buffered saline (PBS) containing 1% BSA and 1% ovalbumin. The pins were then incubated overnight at 4°C in the primary antibody diluted in the above PBS solution, followed by washing with PBS containing 0.05% TWEEN 20. The pins were then incubated with the appropriate horseradish peroxidase labeled conjugate, washed and developed with colorimetric detection as described above.

Identification of the Critical Region of the IFN-γ Receptor

An anti-idiotypic antibody was used to carry out analyses to identify the critical region of the human IFN-γ receptor. This antibody, which was prepared against an IgG antibody fraction specific for a polypeptide having an amino acid sequence corresponding to that of a region of human IFN-γ, mimics IFN-γ itself and thereby specifically binds to the IFN-γ receptor. A complete description of the anti-idiotypic antibody can be found in International Application Publication No. WO 92/06115.

The analysis was carried out by first synthesizing polypeptide octamers corresponding to continuously overlapping regions of the human IFN-γ receptor, and then through a standard ELISA determining which of the octamers bound to the anti-idiotypic antibody.

Overlapping octamer polypeptides were synthesized on polyethylene pins in a 96-pin format using the method of Geysen *et al.* [*Proc. Natl. Acad. Sci.* USA *81*:3998 (1984); *Proc. Natl. Acad. Sci.* USA *82*:178 (19845)]. The polypeptides were synthesized using Fmoc/t-butyl protecting groups and the amino acids being coupled were highly activated pentafluorophenyl and oxo-benzotriazine esters. Approximately 20 to 50 pmoles of peptide were estimated to be synthesized on each pin.

Based upon the foregoing analyses, a number of polypeptides having amino acid sequences corresponding to that of the critical region of the human IFN-γ receptor were synthesized.

Polypeptides

Polypeptides having amino acid sequences defined by SEQ ID NOs: 5-8 were synthesized using the solid-phase

method of Merrifield [*J. Am. Chem. Soc. 85*:2149 (1963)]. An Applied Biosystems (Foster City, CA) Model 430A solid-phase peptide synthesizer was used with t-butyloxycarbonyl chemistry, and the polypeptides were built upon a PAM resin. Hydrogen fluoride was used to cleave the polypeptides from the resin, after which the polypeptides were purified on a PHARMACIA FPLC using a 20 ml Pep/RPC column with a reverse phase chromatography solvent system of TFA/acetonitrile.

The cysteine group of some of the polypeptide defined by SEQ ID NO: 5 was modified by standard methods with acetamidomethyl protecting groups, which were not removed. Some of the data described below were produced with this sulfhydryl-blocked polypeptide.

Amino acid sequencing by automated Edman degradation confirmed the sequences of the polypeptides. FAB mass spectral analysis was carried out on a VG ZAB-SE double focusing mass spectrometer operating at an accelerating voltage of 8 kV. Circular dichroism measurements were made on an IBM-interfaced Jasco 500C spectropolarimeter at room temperature using 1.0 cm path length cells on a protein concentration of 1.0 mg/ml.

Anti-Polypeptide Antibodies

Antibodies against the polypeptides having sequences defined by SEQ ID NOs: 5 (with and without sulfhydryl block) and 6-8 were produced in New Zealand White rabbits (Hazelton Labs) by intradermal immunization with 500 µl volumes (0.1 ml per injected site) of aqueous pH 7.1 solutions containing 0.5 to 1.0 mg of the various polypeptides emulsified with equal volumes of Freund's complete adjuvant. Booster injections containing about 0.25 to 0.5 mg of polypeptide in Freund's incomplete adjuvant were

administered at approximately 4-week intervals as required, as judged by ELISA responses to the polypeptides and to the human IFN-γ receptor.

ELISA of the antisera thus produced showed that all of the antibodies tested were reactive against the polypeptide antigens used to elicit production of the antibodies. The antibodies against the polypeptides having sequences defined by SEQ ID NOs: 5 (with blocked sulfhydryl group) and 8 also bound to the IFN-γ receptor. Presumably, the antibodies against the other polypeptides would also have bound to the receptor, although this was not determined experimentally.

Inhibition of HLA-DR Induction

Determination of the effects of polypeptide antagonists on the induction of HLA-DR antigen expression by IFN-γ was quantified essentially as described by Gibson *et al.* [*J. Immunol. Meth. 125*:103 (1989)]. Briefly, control culture medium and various dilutions in culture medium of the polypeptide defined by SEQ ID NO:5 (blocked at the Cys sulfhydryl group) were incubated in the presence of a fixed concentration (150 pM) of the interferon in 0.1 ml volumes in microtiter plate wells for one hour at 37°C.

Following this incubation, the medium was removed from each well and the wells were washed three times with culture medium. Aliquots (0.1 ml) of culture medium were added to the wells, and the plates were incubated for 48 hours at 37°C to allow induction of HLA-DR antigen expression by the IFN-γ.

The wells were washed with 0.2 ml of phosphate buffered saline (PBS; 0.02 M sodium phosphate, 0.15 M NaCl, pH 7.4) and then fixed for two minutes with ice-cold

anhydrous ethyl alcohol. The alcohol was removed, and the wells were washed once with 0.2 ml of PBS. Fifty microliters of a 1:50 dilution of the mouse monoclonal anti-HLA-DR antibody in PBS containing 0.5% bovine serum albumin were then added to each well, and the plates were incubated for one hour at room temperature.

Excess reagent was removed by washing the wells three times with 0.2 ml of PBS, after which 0.1 ml of a 1:5,000 dilution of peroxidase-labeled goat anti-mouse IgG was added to each well. The plates were incubated for one hour at room temperature. After washing each well three times with PBS as before, color was developed by the addition of ABTS for 5-10 minutes at room temperature. Absorbance was measured at 405 nm using an ELISA plate reader.

Results produced with the polypeptide defined by SEQ ID NO: 5 (blocked at the Cys sulfhydryl group) are shown in Fig. 1, where it can be seen that increasing concentrations of the polypeptide antagonist of from about 10 to 100 μM produced progressively increasing inhibition of HLA-DR antigen expression. At the higher concentrations, the antagonist produced essentially complete inhibition.

Although not actually tested, it would be expected that polypeptides having sequences defined by SEQ ID NOs: 2, 5 (unblocked sulfhydryl group), 6, 7 and 8 would have similar activity.

To determine whether the inhibition observed in Fig. 1 was the result of binding of the polypeptide antagonist to the IFN-γ, 0.1 ml aliquots of a 100 pM solution of the polypeptide were coated onto the wells of a microtiter plate and the plate was blocked with 1% BSA. Varying amounts of IFN-γ were then added to the wells and the plates were incubated and analyzed by ELIZA as described above.

Specifically bound IFN-γ was detected colorimetrically at 405 nm using a neutralizing rabbit anti-human IFN-γ antibody.

The results are shown in Fig. 2, where it can be seen (filled squares) that there was a dose-dependent binding of the human IFN-γ to the immobilized polypeptide, until a saturation plateau was reached. When an unrelated polypeptide was instead first coated onto the wells of the plate (open squares), no IFN-γ binding was observed.

Specific binding of the polypeptide to human IFN-γ was also confirmed by nuclear magnetic resonance (NMR) analysis. NMR spectra of the free polypeptide collected in 20 mM phosphate, pH 7.0, at 5°C with a polypeptide concentration of 7.0 mg/ml (2.66 mM) showed that the polypeptide alone had very little secondary structure. In contrast, NMR analysis of the polypeptide (1.0 mg/ml; 0.38 mM) in the presence of recombinant human IFN-γ E (6.7 mg/ml; 0.20 mM) in the same buffer at 5°C produced a Nuclear Overhauser Effect spectrum indicative of specific binding of the polypeptide to the IFN-γ.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will become apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Schering Corp.

(ii) TITLE OF INVENTION: Antagonists of Human Gamma Interferon

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Schering-Plough Corporation

(B) STREET: One Giralda Farms

(C) CITY: Madison

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07940

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Macintosh 6.0.8

(D) SOFTWARE: Microsoft Word 4.00B

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA: None

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Lunn, Paul G.

(B) REGISTRATION NUMBER: 32,743

(C) REFERENCE/DOCKET NUMBER: JB0285Q

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-822-7255

(B) TELEFAX: 201-822-7039

(C) TELEX: 219165

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 489 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Ala Leu Leu Phe Leu Leu Pro Leu Val Met Gln Gly Val Ser Arg
1               5                  10                  15
Ala Glu Met Gly Thr Ala Asp Leu Gly Pro Ser Ser Val Pro Thr Pro
            20                  25                  30
Thr Asn Val Thr Ile Glu Ser Tyr Asn Met Asn Pro Ile Val Tyr Trp
        35                  40                  45
Glu Tyr Gln Ile Met Pro Gln Val Pro Val Phe Thr Val Glu Val Lys
    50                  55                  60
Asn Tyr Gly Val Lys Asn Ser Glu Trp Ile Asp Ala Cys Ile Asn Ile
65                  70                  75                  80
Ser His His Tyr Cys Asn Ile Ser Asp His Val Gly Asp Pro Ser Asn
                85                  90                  95
Ser Leu Trp Val Arg Val Lys Ala Arg Val Gly Gln Lys Glu Ser Ala
            100                 105                 110
Tyr Ala Lys Ser Glu Glu Phe Ala Val Cys Arg Asp Gly Lys Ile Gly
        115                 120                 125
Pro Pro Lys Leu Asp Ile Arg Lys Glu Glu Lys Gln Ile Met Ile Asp
    130                 135                 140
Ile Phe His Pro Ser Val Phe Val Asn Gly Asp Glu Gln Asp Val Asp
145                 150                 155                 160
Tyr Asp Pro Glu Thr Thr Cys Tyr Ile Arg Val Tyr Asn Val Tyr Val
                165                 170                 175
Arg Met Asn Gly Ser Glu Ile Gln Tyr Lys Ile Leu Thr Gln Lys Glu
            180                 185                 190
Asp Asp Cys Asp Glu Ile Gln Cys Gln Leu Ala Ile Pro Val Ser Ser
        195                 200                 205
Leu Asn Ser Gln Tyr Cys Val Ser Ala Glu Gly Val Leu His Val Trp
    210                 215                 220
Gly Val Thr Thr Glu Lys Ser Lys Glu Val Cys Ile Thr Ile Phe Asn
```

225 230 235 240
Ser Ser Ile Lys Gly Ser Leu Trp Ile Pro Val Val Ala Ala Leu Leu
245 250 255
Leu Phe Leu Val Leu Ser Leu Val Phe Ile Cys Phe Tyr Ile Lys Lys
260 265 270
Ile Asn Pro Leu Lys Glu Lys Ser Ile Ile Leu Pro Lys Ser Leu Ile
275 280 285
Ser Val Val Arg Ser Ala Thr Leu Glu Thr Lys Pro Glu Ser Lys Tyr
290 295 300
Val Ser Leu Ile Thr Ser Tyr Gln Pro Phe Ser Leu Glu Lys Glu Val
305 310 315 320
Val Cys Glu Glu Pro Leu Ser Pro Ala Thr Val Pro Gly Met His Thr
325 330 335
Glu Asp Asn Pro Gly Lys Val Glu His Thr Glu Glu Leu Ser Ser Ile
340 345 350
Thr Glu Val Val Thr Thr Glu Glu Asn Ile Pro Asp Val Val Pro Gly
355 360 365
Ser His Leu Thr Pro Ile Glu Arg Glu Ser Ser Ser Pro Leu Ser Ser
370 375 380
Asn Gln Ser Glu Pro Gly Ser Ile Ala Leu Asn Ser Tyr His Ser Arg
385 390 395 400
Asn Cys Ser Glu Ser Asp His Ser Arg Asn Gly Phe Asp Thr Asp Ser
405 410 415
Ser Cys Leu Glu Ser His Ser Ser Leu Ser Asp Ser Glu Phe Pro Pro
420 425 430
Asn Asn Lys Gly Glu Ile Lys Thr Glu Gly Gln Glu Leu Ile Thr Val
435 440 445
Ile Lys Ala Pro Thr Ser Phe Gly Tyr Asp Lys Pro His Val Leu Val
450 455 460
Asp Leu Leu Val Asp Asp Ser Gly Lys Glu Ser Leu Ile Gly Tyr Arg
465 470 475 480
Pro Thr Glu Asp Ser Lys Glu Phe Ser
485

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Ala Val Cys Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg
1               5                  10                  15
Lys Glu Glu Lys Gln Ile Met Ile Asp Ile Phe His Pro Ser Val Phe
            20                  25                  30
Val Asn Gly Asp Glu Gln Asp Val Asp Tyr Asp Pro Glu Thr Thr Cys
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ala Xaa Xaa Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg
1 5 10 15
Lys Glu Glu Lys Gln Ile
20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ala Xaa Xaa Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg
1 5 10 15
Lys Glu Glu Lys Gln Ile Met Ile Asp Ile Phe His Pro Ser Val Phe
20 25 30
Val Asn Gly Asp Glu Gln Asp Val Asp Tyr Asp Pro Glu Thr Thr Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Ala Tyr Cys Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg
1               5                   10                  15
Lys Glu Glu Lys Gln Ile
            20
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Ala Val Cys Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg
1               5                   10                  15
Lys Glu Glu Lys Gln Ile
            20
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Ala Val Ser Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg
1               5                  10                  15
Lys Glu Glu Lys Gln Ile
            20
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Arg Asp Gly Lys Ile Gly Pro Pro Lys Leu Asp Ile Arg Lys Glu Glu
1               5                  10                  15
```

WHAT IS CLAIMED IS:

1. An antagonist of human IFN-γ that mimics, comprises or specifically binds to an amino acid sequence of a region of the human IFN-γ receptor, which region has an amino acid sequence defined by the sequence of SEQ ID NO: 2.

2. The antagonist of claim 1 which is a polypeptide that contains a core sequence defined by SEQ ID NO: 3 and comprises from about 22 to 48 amino acid residues of the amino acid sequence defined by SEQ ID NO: 4, wherein residues represented as Xaa at positions 2 and 3 can be Tyr or Val and Ser or Cys, respectively, the sulfhydryl groups of which Cys residues can be free or blocked by a sulfhydryl blocking group.

3. The polypeptide of claim 2 which has an amino acid sequence defined by SEQ ID NO: 2, 5, 6 or 7.

4. The antagonist of claim 1 which is an antibody or a fragment thereof that specifically binds to an epitope of a polypeptide having an amino acid sequence defined by part or all of the sequence of SEQ ID NO: 2, and to the human IFN-γ receptor.

5. The antagonist of claim 4 which specifically binds to a polypeptide having an amino acid sequence defined by SEQ ID NO: 2, 5, 6, 7 or 8.

6. The antagonist of claim 1 which is an anti-idiotypic antibody or a fragment thereof produced against an antibody or a fragment thereof that specifically binds to an epitope of a polypeptide having an amino acid sequence defined by part or all of the sequence of SEQ ID NO: 2, and to the human IFN-γ receptor.

7. A method for inhibiting the biological activity of human IFN-γ comprising contacting human IFN-γ or cells bearing receptors for human IFN-γ with an antagonist of human IFN-γ that mimics, comprises or specifically binds to an amino acid sequence of a region of the human IFN-γ receptor, which region has an amino acid sequence defined by the sequence of SEQ ID NO: 2.

8. The method of claim 7 in which the antagonist is a polypeptide that contains a core sequence defined by SEQ ID NO: 3 and comprises from about 22 to 48 amino acid residues of the amino acid sequence defined by SEQ ID NO: 4, wherein residues represented as Xaa at positions 2 and 3 can be Tyr or Val and Ser or Cys, respectively, the sulfhydryl groups of which Cys residues can be free or blocked by a sulfhydryl blocking group.

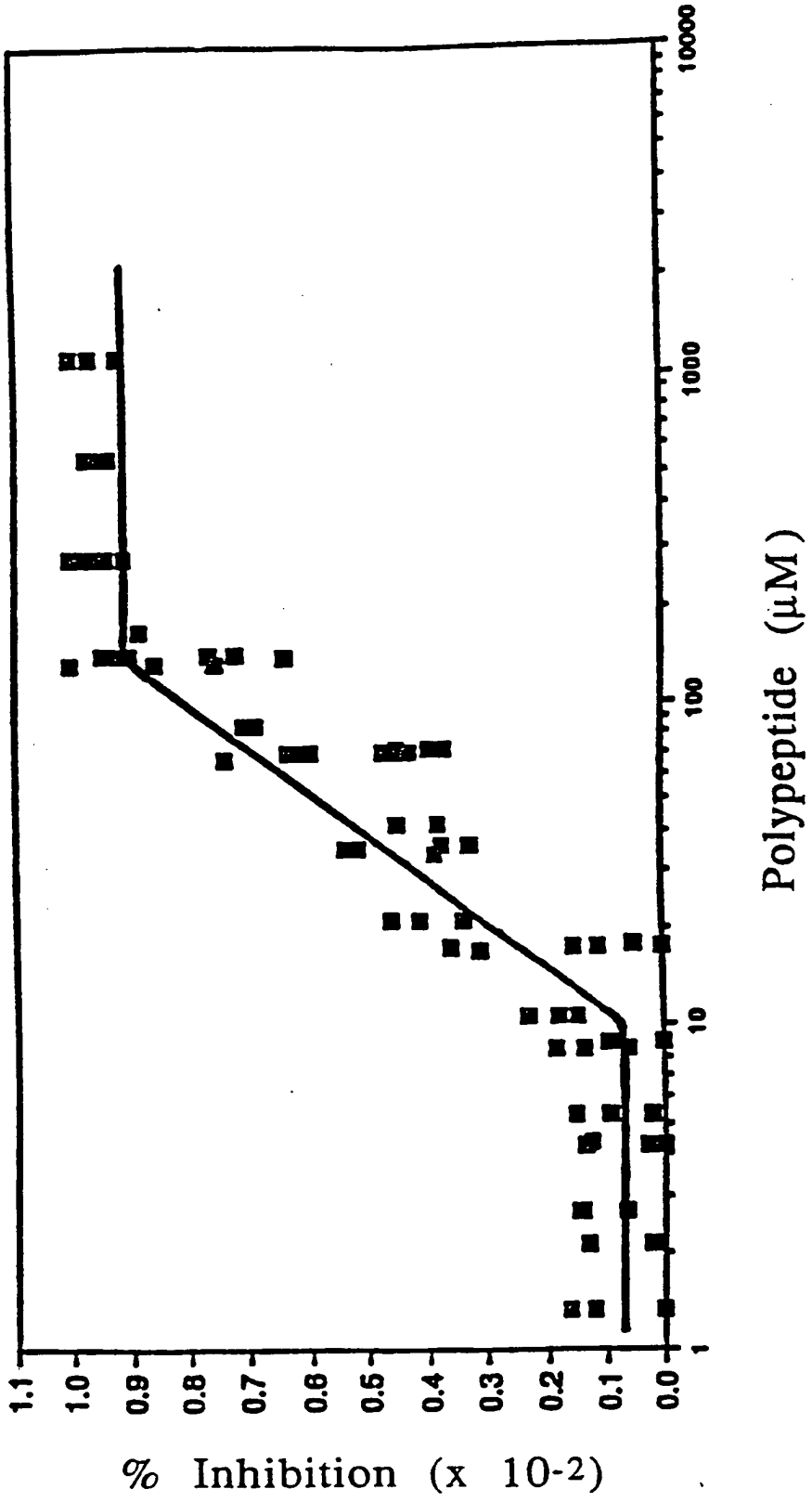
9. The method of claim 8 in which the polypeptide has an amino acid sequence defined by SEQ ID NO: 2, 5, 6 or 7.

10. The method of claim 7 in which the antagonist is an antibody or a fragment thereof that specifically binds to an epitope of a polypeptide having an amino acid sequence defined by part or all of a sequence defined by SEQ ID NO: 2, 5, 6 or 7, and to the human IFN-γ receptor.

11. The method of claim 10 in which the polypeptide has an amino acid sequence defined by SEQ ID NO: 2, 5, 6, 7 or 8.

12. The method of claim 7 in which the antagonist is an anti-idiotypic antibody or a fragment thereof produced against an antibody or a fragment thereof that specifically binds to an epitope of a polypeptide having an amino acid sequence defined by part or all of the sequence of SEQ ID NO: 2, and to the human IFN-γ receptor.

Fig. 1


10000
1000
100
10
1
Polypeptide (μM)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
% Inhibition (x 10-2)

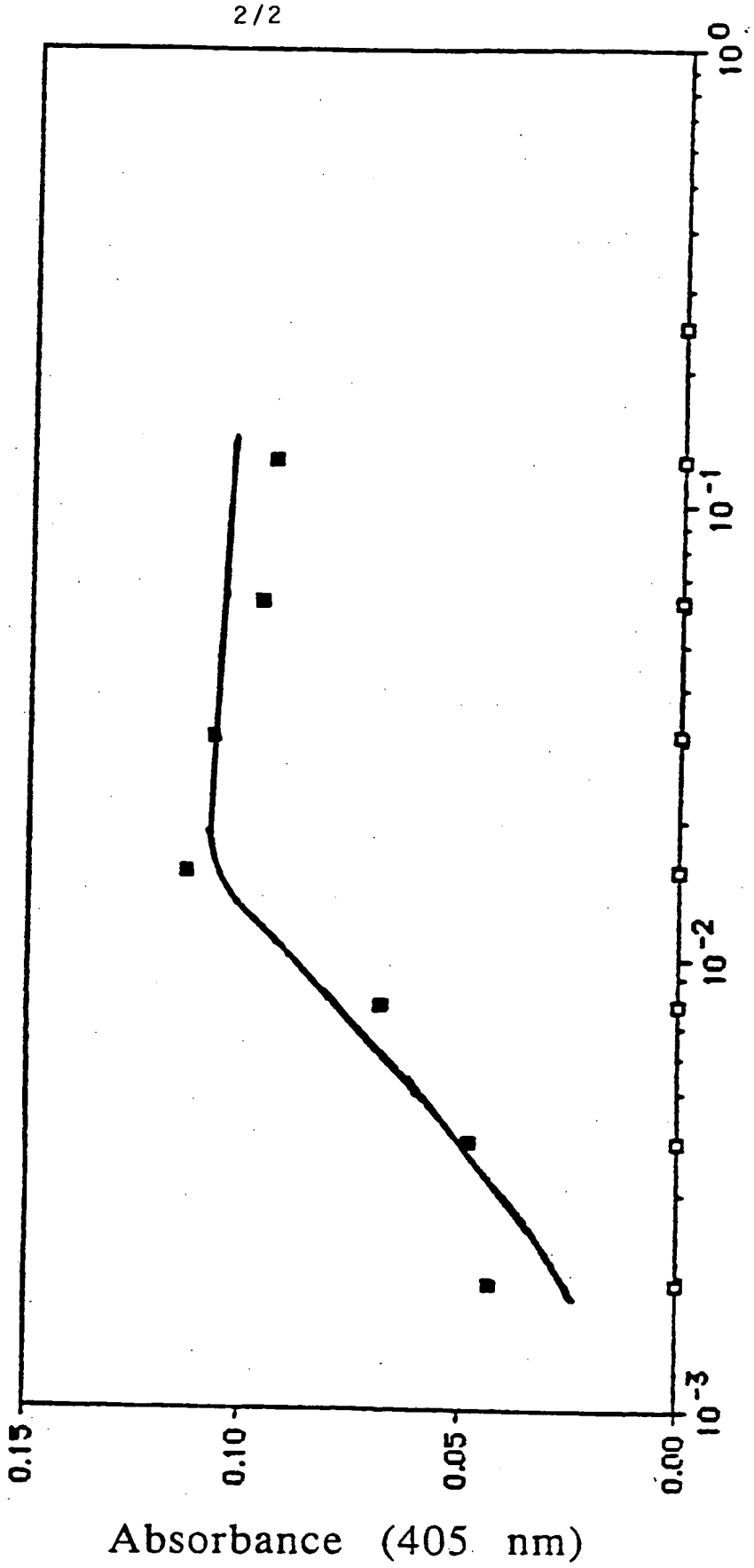


Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/11110

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/10; C07K15/06; A61K37/02; A61K39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP,A,0 393 502 (HOFFMANN-LA ROCHE) 24 October 1990 * claims 1-3, 13,16; figs 82-87 * ---	1-3,7-9
X	EP,A,0 416 652 (YEDA) 13 March 1991 * page 2; claim 1 * ---	1-3,7-9
X	WO,A,9 116 431 (SCHERING) 31 October 1991 * page 4, lines 7-18 * ---	1-3,7-9
X	WO,A,9 206 115 (SCHERING) 16 April 1992 cited in the application * claims 3-10 * ---	4-6, 10-12

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 FEBRUARY 1994	25 -03- 1994
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HERMANN R.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	J.BIOL.CHEM. vol. 265, no. 12, 25 April 1990, pages 6908 - 6915 GAROTTA, G. ET AL. 'Human interferon-gamma receptor' * page 6912 *	1-5,7-11
T	PHARMACOLOGICAL RESEARCH vol. 21, no. 2, 1989, pages 5 - 17 GAROTTA, G. ET AL. 'Development of interferon-gamma antagonists ...' * page 13, paragraph entitled "IFNgamma receptor" *	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9311110
SA 82579

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 23/02/94

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0393502	24-10-90	AU-B- 625866	16-07-92
		AU-A- 5309390	08-11-90
		CA-A- 2014752	19-10-90
		JP-A- 2303490	17-12-90
EP-A-0416652	13-03-91	AU-A- 6220690	14-03-91
		CA-A- 2024767	08-03-91
		JP-A- 3204896	06-09-91
		US-A- 5221789	22-06-93
WO-A-9116431	31-10-91	AU-A- 7778991	11-11-91
		CN-A- 1056125	13-11-91
		EP-A- 0528847	03-03-93
		JP-T- 5502037	15-04-93
WO-A-9206115	16-04-92	AU-A- 8742991	28-04-92
		CA-A- 2092541	28-03-92
		EP-A- 0550650	14-07-93